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THE EFFECT OF SODIUM FLUORIDE ON THE GROWTH AND SURVIVAL OF SOME BACTERIAL SPECIES IMPORTANT IN WATER QUALITY MEASUREMENT

DIVISION OF RESEARCH
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INTRODUCTION

Although studies on the effect of fluoride ions have been carried out with fish and lobsters (1,7,10) using concentrations up to 200 ppm, there appear to be few documented reports regarding their effect on the bacterial population in water. Kniznikov (4) completed an investigation with the intestinal dysentery bacteria and reported that fluorine in quantities normally found in drinking water, had no effect on the metabolism, morphology or antigenicity of the organisms over an extended period of time. However, with the greater use of reclaimed waste waters, fluoride concentrations could conceivably increase significantly. The object of this study was therefore to determine the effects of various concentrations of sodium fluoride upon the growth and survival of several species of bacteria, namely Escherichia (E.) coli, Pseudomonas (Ps.) fluorescens and Enterococcus (faecal streptococcus). These are indicator organisms and thus of prime importance in the determination of water quality.

MATERIALS AND METHODS

Media Difco Bacto Nutrient agar, Nutrient broth and Tryptone Glucose Extract agar, prepared according to directions, were employed.

Davis medium (3), was slightly modified and prepared as follows: $K2HPO_4$-7 gm., KH_2PO_4-3 gm., sodium citrate $3H_2O$-0.5 gm., $MgSO_4.7H_2O$-0.1 gm., $(NH_4)_2SO_4$-1 gm., dissolved in 900 ml. distilled water and autoclaved at 15 psi. for 15 minutes, after adjusting the pH to 7.0 with N/10 NaOH. Glucose (2 gm.) was added to 100 ml. distilled water and autoclaved at 15 psi for 15 minutes. The two solutions were mixed aseptically before dispensing the medium in the required amounts into sterile containers.

The sodium fluoride was made up as stock solutions containing 1 gm. or 0.1 gm. sodium fluoride per 100 ml. distilled water. The solutions were autoclaved at 15 psi. for 15 minutes and were added aseptically to the media prior to use, to give the required sodium fluoride concentration. After autoclaving, the sodium fluoride concentration in the solutions was checked by standard chemical means and found to be correct. The sodium fluoride was added to nutrient broth or Davis medium in the growth experiments, and to distilled water for the determinations of the viability on storage.

For all experiments, 30 ml. of test medium was added to a 50 ml.-capacity, screw-cap culture tube.

Cultures The strains of E.coli, Ps.fluorescens and Enterococcus were obtained from the Bacteriology Branch of the Laboratory Division of the OWRC, having been originally isolated from routine water samples. They were maintained on nutrient agar slopes (E.coli and Ps.fluorescens), or tryptone glucose extract agar slopes (Enterococcus), grown aerobically at room temperature. Cells for the experiments were grown in nutrient broth, inoculated from a slant culture grown for 24 hours at 35°C. Inocula were adjusted by dilution to the required level, by reference to a curve relating the turbidity of a culture to the number of organisms per ml.; turbidity was measured as percent transmittance of the culture in a Spectronic 20 (Bausch & Lomb) spectrophotometer at 550 λ, compared to a control of medium containing no cells. The viable number was determined by plate count (11) using nutrient agar.

EXPERIMENTAL

The effect of sodium fluoride on growth The effect of sodium fluoride concentrations of 1,5,10,50,100 and 200 ppm. on the growth of equal inocula (10^7 cells per culture) in broth, was determined in terms of turbidity compared to a control culture containing no sodium fluoride. Three separate cultures were set up for each organism, at each test concentration, and measurements were made after growth at 35°C for 24 and 48 hours. Another complete set of tests was carried out using room temperature incubation.

At the same time as turbidometric measurements were made, the cells in each culture were checked morphologically by preparing slides and staining with Gram's stain.

Since the fluoride ions may be inactivated by combination with organic constituents in the broth medium, a mineral medium (Davis), capable of supporting the growth of E.coli and Ps.fluorescens was also employed. In this case, sodium fluoride concentrations of 5,50,200,600 and 800 ppm were tested in the same way as before, but incubating duplicate cultures at 35°C only; the control tubes contained no added fluoride. The morphology of all cultures was also determined after 48 hours.

The effect of sodium fluoride on viability Approximately equal numbers (to yield about 10^6 cells per ml.) of the three species were inoculated into separate series of distilled water tubes containing 1,5,10,50,100 and 200 ppm sodium fluoride. At monthly intervals, the number of viable bacteria remaining at each fluoride level was determined by plate count. The morphology of the colonies and cells grown in the plate counts was examined. The viability of cells in the various test solutions was thus compared to that in controls, which contained no sodium fluoride. The complete test was carried out in duplicate.

RESULTS AND DISCUSSION

The effect of various concentrations of sodium fluoride on the growth of bacteria in nutrient broth after 48 hours is shown in Table 1. Although fluoride ions are reported to inhibit respiratory enzymes, at least in the higher plants (5), there was no significant difference observed between the growth of cultures containing sodium fluoride and corresponding control tubes, at either of the growth temperatures employed after growth for 48 hours. Also, there appeared to be no morphological difference between control cultures and cells grown in the presence of sodium fluoride; furthermore, there was no change in the Gram reaction of any of the cultures.

It is possible that the cells grown in the presence of fluoride ions may have differed metabolically from those in the control cultures, but no biochemical studies were undertaken.

In the mineral medium, significantly less growth occurred at 48 hours, only at levels of sodium fluoride approaching 800 ppm (Table 2.) However, morphologically identical cells were harvested from all tubes. It is interesting to note (6), that sodium fluoride in amounts approximating to 400 ppm had no inhibiting effect on the basal metabolism of oral flora under aerobic conditions; however, anaerobically, a 40% inhibition of basal metabolism occurred.

When the viability of the bacterial cells in the various concentrations of sodium fluoride was compared to that

in the controls, no significant difference was found after 4 months storage, even at 200 ppm (Table 3). The morphology of the colonies and cells grown from the tubes at various times, was apparently normal, although this could have been due to a reversion occuring on transfer to normal medium.

TABLE 1

Transmittance of broth cultures of E.coli, Ps.fluorescens and Enterococcus after growth for 48 hours at 35°C or room temperature, in the presence of various amounts of sodium fluoride.

Conc. NaFl ppm	Percent Transmittance					
	E.coli		Ps.fluorescens		Enterococcus	
	RT*	35°C	RT	35°C	RT	35°C
0 (Control)	20**	5.5	20	4	68	60
1	38	0***	16	0	67	60
5	33	4.5	18	2	68	62
10	23	0	12	1	68	61
50	27	4	9	4	67	62
100	28	3.5	13	7	69	64
200	30	2.0	11	4	63	67

* room temperature

** the results are the means of 3 cultures

*** turbidity of culture beyond the limits of measurement

TABLE 2

Transmittance of cultures of E.coli and Ps.fluorescens grown in Davis medium for 48 hours at 35°C in the presence of various amounts of sodium fluoride.

Conc. NaFl ppm	Percent Transmittance	
	E.coli	Ps.fluorescens
0	0*	54
5	0	50
50	0	50
200	5	56
600	40	58
800	55	73

* the results are the means of duplicate cultures.

TABLE 3

Bacteria Surviving in distilled water containing 200 ppm sodium fluoride after storage at room temperature for periods up to 4 months.

Time of Sample	E.coli		Ps.Flucrescens		Enterococcus	
	Control	200ppmNaFl	Control	200ppmNaFl	Control	200ppmNafl
Initial	$1.3x10^6$*	$1.3x10^6$	$1.4x10^6$	$1.4x10^6$	$5.3x10^5$	$5.3x10^5$
1 Month	$1.2x10^7$	$9.2x10^6$	$1.9x10^7$	$8.4x10^6$	$3.3x10^3$	$2.0x10^2$
2 Months	$1.1x10^7$	$6.8x10^6$	$1.6x10^7$	$1.1x10^7$	$7.6x10^2$	$3.0x10^1$
3 Months	$3.4x10^6$	$5.3x10^5$	$7.7x10^6$	$9.6x10^1$	$6.5x10^1$	$1.0x10^1$
4 Months	$9.7x10^5$	$3.2x10^5$	$3.6x10^6$	$3.7x10^6$	$6.0x10^1$	$1.2x10^1$

* the results are the means of duplicate tubes.

CONCLUSIONS

Sodium fluoride has no effect on the growth, morphology or viability of the three species of bacteria, except possibly at concentrations approaching 800 ppm. Since this concentration is far in excess of any expected in natural or treated waters, even if the recycling of waste waters were carried out repeatedly, it is unlikely that the fluoridation of water would have any direct effect on the bacteria used as indicators of water quality.

Several other interesting points emerge from this study. When the distilled water systems were inoculated, a small amount of organic material would have been added. After the first week of storage, both E.coli and Ps.fluorescens showed an increase in numbers over those originally added (approximately 10 times). Over the same period of time the numbers of Enterococci had already decreased; this is in accord with the findings of Slanetz and Bartley (9), when Enterococci did not increase in numbers in water (sea water) containing organic matter, whereas Ps.fluorescens and E.coli did multiply. Other studies (12) have demonstrated multiplication of E.coli in the presence of very low concentrations of organic materials in water. This multiplication could lead to erroneously high estimates of the pollution in such waters, if either of these organisms were used as an indicator.

Furthermore, Ps.fluorescens and E.coli numbers, after four months, had scarcely fallen below their original level, whereas Enterococci had decreased to less than 100 organisms per ml. during the same time. Enterococci are thus not good indicators of distant or previous pollution; conversely, even if pollution were to occur for only a short period, or if it were intermittent, E.coli or Ps.fluorescens counts would give the impression of continuous high level pollution although the waters may be free of extraneous organic matter. The use of only one organism as an indicator of pollution, as reported by other workers (8,2) may thus be of doubtful value, in the case of certain types of water samples.

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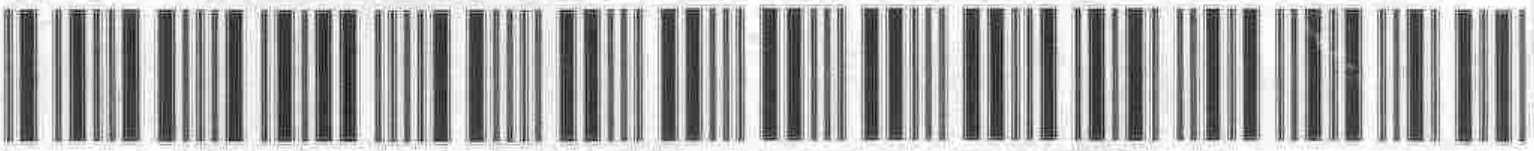
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